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THE METABOLISM OF "LEPRA BACILLUS," GRASS
BACILLUS, AND SMEGMA BACILLUS IN
PLAIN, DEXTROSE, MANNITE, AND
GLYCERIN BROTHS

STUDIES IN ACID-FAST BACTERIA. V.*

A. I. KENDALL, A. A. DAY AND A. W. WALKER

(From the Department of Bacteriology, Northwestern University Medical School, Chicago.)

The study of the metabolism of two avirulent, rapidly growing strains of the human tubercle bacillus in plain, dextrose, mannite, and glycerin broth, respectively, showed two distinct phases in the development of the culture; an initial phase, in which the morphological picture was characterized by the development of a considerable proportion of non-acid-fast bacilli, associated with a progressive increase in the breakdown of the protein constituents of the medium, as shown by the increased formation of ammonia. This initial phase, which reached its maximum at about the third week, was succeeded by a second, recessive phase, in which all of the bacteria were acid-fast, and in which, furthermore, the ammonia which had accumulated during the initial phase gradually decreased, or at least gradually became undetectable by the method used (the Folin air current method), until finally it had practically disappeared. This same phenomenon was observed when these organisms were grown in simpler media; even in a medium as simple in composition as di-ammonium hydrogen phosphate as a combined source of nitrogen and phosphorus, with dextrose as a source of carbon, and NaCl to maintain the proper osmotic pressure of the solution.

The question arises, is this somewhat unusual series of events met with in cultures of other acid-fast bacteria, not tubercle bacilli, grown under the same conditions? If this recession of ammonia is a feature of the growth of the majority, or all acid-fast bacilli in broth, it would appear to differentiate these organisms somewhat sharply from other non-acid-fast bacteria, for the latter organisms either do not exhibit this phenomenon, or exhibit it to a lesser degree.

With this possibility in view, the metabolism of three representative types of the acid-fast group of bacteria, the grass bacillus, the smegma

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TABLE 1
METABOLISM OF *LEPRA BACILLUS* (DUVAL)

Mannite Broth						Glycerin Broth				
Days	Alizarin	Neutral Red	Phenolphthalein	NH ₃ mg. Increase per 100 c.c. Broth	NH ₃ Total N ₂ Percent	Alizarin	Neutral Red	Phenolphthalein	NH ₃ mg. Increase per 100 c.c. Broth	NH ₃ Total N ₂ Percent
1	0.00	-0.30	-0.10	+9.8	+3.04	-0.20	-0.10	0.00	1.4	0.44
3	+0.10	-0.20	-0.30	0.00	0.00	+0.20	+0.10	-0.10	1.4	0.44
10	-0.20	-0.30	-0.80	-4.9	-1.52	-0.40	-0.10	-0.50	-0.7	-0.22
21	-0.10	-0.50	-0.90	-4.9	-1.52	-1.80	-1.00	-0.80	-5.6	-1.74
28	-0.40	-0.80	-0.60	-9.8	-3.04	-1.70	-1.10	-1.30	-9.1	-2.82
43	-1.20	-1.80	-1.40	-6.3	-1.95	-1.30	-1.40	-1.30	-11.2	-3.48
51	-1.60	-1.40	-1.70	-13.3	-4.13

TABLE 2
METABOLISM OF *GRASS BACILLUS* III.

Plain Broth						Dextrose Broth				
Days	Alizarin	Neutral Red	Phenolphthalein	NH ₃ mg. Increase per 100 c.c. Broth	NH ₃ Total N ₂ Percent	Alizarin	Neutral Red	Phenolphthalein	NH ₃ mg. Increase per 100 c.c. Broth	NH ₃ Total N ₂ Percent
1	-0.10	-0.10	-0.10	-0.7	-0.22	-0.90	-0.20	-0.70	9.8	3.04
3	-0.30	-0.40	-0.20	1.4	0.44	-0.80	-0.30	-0.90	-0.7	-0.22
6	-1.00	-0.40	-1.10	4.2	1.30	-2.20	-0.90	-1.80	-0.7	-0.22
10	-2.70	-1.40	-1.70	14.7	4.57	-2.90	-1.20	-1.60	5.6	1.74
15	-1.90	-2.10	-1.50	24.5	7.60	-3.50	-2.80	-2.00	23.8	7.40
21	-3.60	-4.00	-2.00	23.8	7.40	-3.50	-2.00	-2.20	21.7	6.75
28	-2.50	-2.00	-1.60	20.3	6.30	-3.50	-2.80	-2.20	16.1	5.00
36	-3.10	-1.30	-1.90	7.0	2.2	-2.80	-2.20	-2.30	8.4	2.60
43	-2.20	-2.10	-1.80	1.4	0.44	-2.50	-2.10	-2.40	1.4	0.44
52	-1.90	-2.00	-2.00	-4.9	-1.52	-2.30	-1.80	-2.10	-4.9	-1.52

TABLE 3
METABOLISM OF *SMEGMA BACILLUS*

Mannite Broth						Glycerin Broth				
Days	Alizarin	Neutral Red	Phenolphthalein	NH ₃ mg. Increase per 100 c.c. Broth	NH ₃ Total N ₂ Percent	Alizarin	Neutral Red	Phenolphthalein	NH ₃ mg. Increase per 100 c.c. Broth	NH ₃ Total N ₂ Percent
1	-0.30	-0.30	-0.30	8.4	2.60	-0.60	-0.20	-0.30	-0.7	-0.22
3	-1.00	-0.50	-0.70	0.00	0.00	-0.10	0.00	-0.20	-0.7	-0.22
6	-1.70	-0.70	-1.30	0.00	0.00	-1.00	-0.40	-0.50	+4.2	1.30
10	-1.40	-0.80	-0.80	1.4	0.44	+0.10	-0.70	-0.30	-2.8	-0.87
15	-1.30	-1.60	-0.50	16.8	5.22	-0.70	-0.40	-0.50	-2.8	0.87
21	-1.50	-1.20	-0.90	16.8	5.22	+1.20	+1.60	+0.60	+12.6	+3.91
28	-2.90	-2.30	-1.50	28.0	8.70	+0.70	+1.10	+0.60	14.0	4.40
36	-2.20	-2.20	-1.80	14.0	4.35	-0.40	-0.80	-0.60	0.7	0.22
43	-2.40	-2.10	-1.60	19.6	6.04	+0.70	-0.60	0.00	0.7	0.22
52	-1.50	-2.20	-2.00	-5.6	-1.74	-0.30	-0.80	-0.40	-4.9	-1.52

METABOLISM OF LEPRA, GRASS, AND SMEGMA BACILLI 441

TABLE 1
METABOLISM OF LEPRA BACILLUS (DUVAL)—(Continued)

Plain Broth						Dextrose Broth				
Days	Alizarin	Neutral Red	Phenolphthalein	NH ₃ mg. Increase per 100 c.c. Broth	NH ₃ Total N ₂ Percent	Alizarin	Neutral Red	Phenolphthalein	NH ₃ mg. Increase per 100 c.c. Broth	NH ₃ Total N ₂ Percent
1	0.00	—0.10	0.00	0.00	0.00	—0.70	—0.30	—0.50	—0.7	—0.22
3	+0.10	0.00	—0.20	0.00	0.00	—0.10	0.00	—0.60	—1.4	—0.44
10	—1.30	—0.60	—1.20	0.00	0.00	—1.30	—0.60	—1.20	0.00	0.00
21	—0.10	—0.40	—0.70	0.7	0.22	—2.00	—1.10	—1.00	—1.4	—0.44
28	—2.30	—1.50	—1.50	—2.8	—0.87	—2.00	—1.00	—0.90	—12.6	—3.92
43	—2.30	—1.90	—2.20	—12.6	—3.92	—2.10	—1.50	—2.20	—16.8	—5.20
51	—1.40	—1.40	—1.90	—11.9	—3.70	—1.50	—1.60	—2.20	—14.7	—4.56

TABLE 2—(Continued)
METABOLISM OF GRASS BACILLUS III

Mannite Broth						Glycerin Broth				
Days	Alizarin	Neutral Red	Phenolphthalein	NH ₃ mg. Increase per 100 c.c. Broth	NH ₃ Total N ₂ Percent	Alizarin	Neutral Red	Phenolphthalein	NH ₃ mg. Increase per 100 c.c. Broth	NH ₃ Total N ₂ Percent
1	+0.10	—0.20	—0.40	9.8	3.04	—0.40	—0.10	—0.10	7.7	2.39
3	+0.10	—0.30	—0.40	0.00	0.00	—0.70	—0.20	—0.30	0.00	0.00
6	—0.10	—0.30	—0.70	0.7	0.22	—1.20	—0.50	—1.00	0.00	0.00
10	—1.10	—0.50	—0.90	1.4	0.44	—0.70	—0.30	—0.40	7.7	2.39
15	—1.50	—1.50	—1.10	8.4	2.60	—0.90	—1.20	—0.30	13.3	4.13
21	—1.50	—1.30	—1.30	15.4	4.78	—0.20	—0.50	—0.30	16.8	5.22
28	—2.10	—1.90	—1.30	11.9	3.70	—1.10	—0.90	+0.30	13.3	4.13
36	—1.90	—1.70	—1.40	16.1	5.00	—0.50	—1.40	+0.10	15.4	4.78
43	—1.80	—1.90	—1.80	2.8	0.88	0.00	—1.20	0.00	4.9	1.52
52	—1.60	—1.70	—1.80	—4.9	—1.52	—0.50	—0.60	—0.20	1.4	0.43

TABLE 3
METABOLISM OF SMEGMA BACILLUS—(Continued)

Plain Broth						Dextrose Broth				
Days	Alizarin	Neutral Red	Phenolphthalein	NH ₃ mg. Increase per 100 c.c. Broth	NH ₃ Total N ₂ Percent	Alizarin	Neutral Red	Phenolphthalein	NH ₃ mg. Increase per 100 c.c. Broth	NH ₃ Total N ₂ Percent
1	—0.80	—0.40	—0.50	9.1	2.83	—1.50	—0.40	—0.90	8.4	2.61
3	—1.20	—0.50	—1.10	0.7	0.22	—1.20	—0.60	—1.10	—0.7	—0.22
6	—1.90	—1.00	—1.90	5.6	1.74
10	—2.20	—1.30	—1.50	11.2	3.48	—2.90	—1.50	—1.10	18.9	5.87
15	—3.60	—2.20	—1.50	19.6	6.08	—3.00	—2.30	—1.10	22.4	6.96
21	—3.40	—1.20	—1.60	7.7	2.39	—4.10	—1.80	—2.10	28.0	8.70
28	—1.50	—1.60	—1.40	7.7	2.39	—3.50	—2.80	—2.10	20.3	6.30
36	—0.70	—1.20	—1.30	0.00	0.00	—3.90	—2.60	—1.20	23.8	7.40
43	—1.70	—1.80	—1.80	—4.2	—1.30	—3.30	—2.40	—2.40	18.2	5.66
52	—2.00	—2.20	—2.20	—7.7	—2.39

bacillus, and the "lepra bacillus" isolated by Duval, were studied in plain, dextrose, mannite, and glycerin nutrient broths. The technic of experimentation throughout was that used for the study of the tubercle bacillus.

The results, shown in tabular form, are self-explanatory in the light of the observations made on the rapidly growing, human tubercle bacilli. The smegma bacillus and the grass bacillus, altho they do not form as much ammonia under parallel conditions as did the tubercle bacilli, present a well-marked maximum followed by a steady decline in the amount of ammonia detectable in the media in which they were grown. The "lepra bacillus" appears to be somewhat different from the grass and smegma bacilli. First, in that the amount of ammonia produced is very slight, the maximum, 1.40 mg. per 100 c.c. broth, being found in the mannite medium at the end of twenty-four hours' incubation. In all media there was actually less ammonia after a few days' incubation than there was in the uninoculated control. This observation agrees with one recorded previously,¹ where, however, the experiment was only carried on for nine days. The cultural reactions and the curve of metabolism of this bacillus would seem to distinguish it rather sharply from the two organisms mentioned above.

SUMMARY

The metabolism of the smegma and grass bacilli resembles that of the rapidly growing, human tubercle bacilli, described previously, in two important particulars; neither dextrose, mannite, nor glycerin exhibits any appreciable sparing action for the protein constituents of the broth, the amounts of ammonia produced being practically the same in these media as in plain broth; and their cultures present a gradual increase in proteolysis to a maximum which is followed by a clearly defined recession of the metabolism indicated by a gradual decrease in the ammonia content.

The "lepra bacillus" does not present this metabolic phenomenon. This would suggest that this bacillus was entirely distinct in its cultural relationships from the grass and smegma bacilli, which follow more closely the metabolism of the tubercle bacillus.

1. Kendall, Day and Walker, *Jour. Am. Chem. Soc.*, 1913, 35, p. 1248.